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Biotreatment for effective degradation and decoloration of textile effluent using novel spore forming *Bacillus* sp

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Bacillus sp
Decoloration

Abstract

Bacillus sp, an effective spore forming organism revealed a result in degrading and decolorizing the textile effluent, which was one of the major environmental pollution in modern world. This work mainly focused in tune with the decolaration of textile effluent sample collected from Salem district. Textile effluent samples were analysed for heavy metals (Nickel and Zinc) in AAS (Atomic absorption spectrometry). Further the soil samples collected from different areas of contaminated with effluent was enriched, isolated and characterized and PCR amplified for species identification and confirmed as *Lysini bacillus sphaericus*. The genus Bacillus, which was beneficial for the degradation of toxic constituents present in the textile effluents, further it was also confirmed by the decolorization bioassay with least value of the final color. The continued development and application of biotechnologies for the biodegradation is limited primarily by physical factors such as pH, temperature and substrate concentration. Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluents. The ubiquitous nature of bacteria makes them invaluable tools in effluent biotreatment. Decoloration of dye from effluent samples, were monitored for 4-8 days by spectrometric observation.

1. Introduction

The textile industry plays an important role in the world economy as well as in our daily life, but at the same time, it consumes large quantities of water and generates huge amounts of wastewaters (Hai *et al.,* 2006). The dyeing industry has been referred as one of the four major polluting industries in India. The Indian textile industry is the world's second largest after China. There are about 1, 200 medium to large-scale textile mills in India, 20% of which are located in Tamil Nadu. Sources also claim about 3, 000 garment manufacturing units employing about 3 million people. All together, India exports to 162 countries, which accounts for 38 percent of India's export ($ 35 billion in 2000). Various reports have mentioned the direct and indirect toxic effects of the dyes and metals that can lead to the formation of tumors, cancers, and allergies besides growth inhibition of Bacteria, protozoan, algae, plants and different animals including human beings (Sponza *et al.,* 2002).Heavy metals are often present in different textile processes and are frequently found in textile dyeing wastewaters are free ionic metals or complex metals, which contribute to environmental concerns (Hill *et al.,* 1993). Industrial production can give rise to saturation levels of cr, cu, zn, pb and Ni in areas close to industrial sites (Nragu *et al.,* 1998) specific studies on evaluating the toxicity caused by textile effluents rich in toxic metals have also been carried out (Spoza *et al,* 2002).Colour is imparted to textile effluents because of various dyes and pigments used (Naeem Ali *et al.,* 2003).

Numerous studies were devoted to elimination of textile effluent, and heavy metals mainly concentrated on the development of an efficient and cost, effective removal process. These include physiochemical methods such as filtration, specific coagulation, use of activated carbon and chemical flocculation. Some of these methods are effective but quite expensive (Maier *et al.,* 2004). Similarly, heavy metals can be removed from industrial waste water by a range of physico-chemical treatment technologies such as precipitation, ion exchange, adsorption, electrochemical processes and membrane processes.

A number of biotechnological approaches have been suggested by recent research as of potential interest in combating this pollution source in an eco-efficient manner (McMullan *et al.,* 2001) Bacteria capable of dye decolorization, either in pure cultures or in consortia, have been reported (Pearce *et al.,* 2003).The utilization of microbial consortia offers considerable advantages over the use of pure cultures in the degradation of synthetic dyes (Junnarkar *et al.,* 2006).

2. Materials and Method

Sample collection

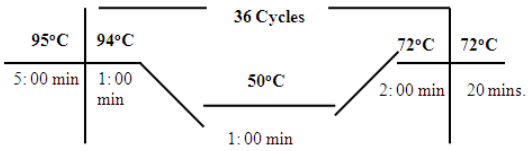
The textile effluent samples were collected from different sites of effluent discharge (Black liquor and final effluent). The soil samples were also collected from different areas around Salem district and effluent discharge area.

Enrichment and isolation

The effluent was enriched with trypticase soy broth overnight and serially diluted and plated on nutrient agar further in Mineral salt medium I containing (Acetic Acid 99.9%; $(NH_2)_2Co$, 100. 0 mg; KH_2PO_4, 67.0 mg; $NaHCO_3$, 840.0 mg; $MgSO4.\ 7H_2O$,38.0 mg;$Cacl_2$ 21.0 mg; $Fecl_3.\ 6H_2O$ 7.0 mg; Glucose – 6.0 gm) were sterilized at 121ºC for 15 min. They were cooled to 50ºC and plated for isolated colonies; further those colonies were characterized for morphology and biochemical characters and extended to species identification through PCR amplification using the primers,

168 RNA gene 8F – 5' – AGA GTT TGA TCC TGG CTC AG 3' and
1490 R – 5' – GAC TTA CCA GGG TAT CTA ATA C – 3' (Sigma Genosys)

Amplification cycle for 16s r RNA gene



Purified PCR products were sequenced using big dye terminator (Applied Bio Systems) in Macrogen Inc., Korea.

Database searching

Nucleotide database was searched with the sequences obtained with NCBI BLAST (Blastn) tool (http://www.ncbi.nlm.nih.gov/BLAST). (Altschul et al., 1997).

Degradation studies

Growth at Different Temperatures

Isolates were plated on mineral salt medium and plated were then incubated at 37^{0}C, 45^{0}C, 65^{0}C, and 70^{0}C. Colony formation was checked periodically up to 4-8 days.

Growth at Different pH Ranges

The ability of the isolates to grow at pH 2, pH 3, pH 5, and pH 6 was tested in Nutrient agar plates. pH was adjusted with 10 NH_2SO_4. The growth was checked periodically upto 4-8 days.

Growth at different substrate concentration

The growth of the microorganisms expose to broad substrate concentration ranging from (25-200). The growth was checked periodically upto 4 - 8 days

Decoloration assay of textile effluent:

The effluent predominately contained a mixture of reactive azodyes. A Loopful of each microbial culture was inoculated for 24h in 10ml culture tubes containing 5 ml nutrient Broth to develop the consortium. A 24h culture of each bacterial isolate (5ml) was added to 250 ml Erlenmeyer flask containing 100 ml of textile effluent (undiluted). The flask was further incubated to observe the time required the decolorization. Aliquots (3ml) of the culture media is were withdrawn at different time intervals, centrifuged at 8000 g for 15 minutes to separate the bacterial cell mass. Decolorization of the textile effluent was analyzed using a UV/Vis spectrophotometer (Hitachi U 2800, Tokyo, Japan) at 490nm. All decolorization experiments were performed in the three sets and the decolorization activity was expressed in terms of the percentage decolorization as follows:

$$\text{Decoloration activity} = \frac{\text{Initial absorbance} - \text{Final Absorbance}}{\text{Initial absorbance}} \times 100$$

3. Results

The results of the study showed that microorganisms *Bacillus sp* is endowed with a high ability of metabolizing the textile effluent (Plate: 1) with the special reference to the heavy metal Zinc, nickel and dye. In this study, the heavy metal utilizing bacteria symbionts were isolated from the textile industry, Salem. The microorganisms responsible for the degradation of heavy metals were identified by the morphological, biochemical and cultural characters.

The heavy metal degrading organisms was isolated from Mineral salt medium. (Plate: 2). Freshly collected soil samples were used for isolation of the microorganisms. The soil samples were plates in nutrient agar medium and incubated for 48-72 hr plate at 40°C. This isolate was selected for further identification, various cultural characterization tests were performed for the selected isolate. From that, the isolate was identified belonging to the genus of *Bacillus* sp. The bacterial species isolated from the soil sample was stained and it showed Gram Positive, Rod shaped colonies under microscope. The isolate showed positive spore formation and it was motile. (Table 1) To determine the phylogeny of bacterial isolate Bacillus sp 16s rRNA gene was amplified and sequenced from isolates.

Table: 1 Morphological characters of Bacillus sp

Characters	Bacillus sp.
Colony property	On nutrient agar, colonies are circular, smooth round, waxy; slight yellow to white, mucoid produces no pigment.
Spores staining	Ellipsoidal and cylindrical, central subterminal, swelling the sporangium.
Gram's staining	Gram positive rod.
Motility	Motile

Analytical techniques

Heavy metal assay was performed by analytical technique by AAS (Atomic absorption spectroscopy). Wherein the results exhibited Nickel was found to be predominant than Zinc. (Table 2 and 3). In addition, the screening of heavy metal (Zinc and Nickel) pertaining to pH was monitored.

Table: 2.Screening of heavy metal Nickel

Concentration(mg/ml)	(PH Value)	
	Control	Sample
25	6.5	6.8
50	6.1	6.3
75	6.0	6.2
100	6.3	6.4
120	5.9	6.1
125	6.1	6.3
140	5.9	6.0
150	4.3	4.5
200	4.8	4.9

Table: 3.Screening of heavy metal Zinc

Concentration(mg/ml)	(PH Value)	
	Control	Sample
25	6.3	6.4
50	7.5	7.8
75	7.0	7.2
100	7.1	7.3
120	7.0	7.1
125	6.9	7.1
6.7	6.7	6.8
150	6.7	6.8
200	7.0	7.1

Degradation studies by varying different parameter

Degradation Studies

Degradation study on heavy metal degradation. The growth of bacterial isolates on the medium was monitored by UV spectrophotometer varying parameter like pH, Temperature, Substrate concentration (Graph 1,2,3, Table 4,5,6)

Table 1; Graph 1: Effect of pH in Degradation of heavy metals using *Bacillus sp*

S. No.	pH	OD value at 600 nm
1	4	0.5
2	5	0.6
3	6	0.8
4	7	1.3
	8	0.9
6	9	0.98

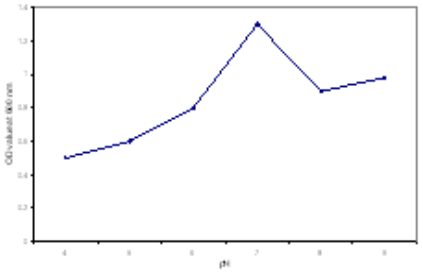


Table 5: Graph 2: Effect of Temperature in Degradation of heavy metals using *Bacillus sp*

S. No	Temperature	OD value at 600 nm
1	25	0.28
2	37	2.8
3	40	1.8

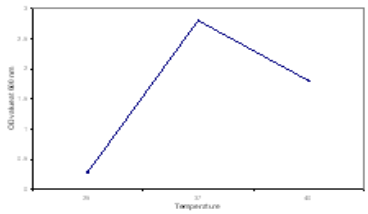


Table 6: Graph 3: Effect of Substrate concentration in Degradation of heavy metals using *Bacillus sp*

S. No	Time	OD Value at 600 nm
1	24	2.83
2	48	4.1
3	72	5.2
4	96	7.2
5	120	9.31

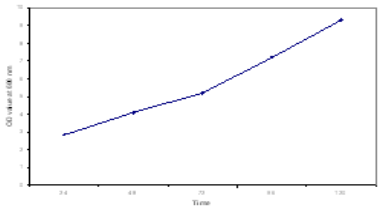
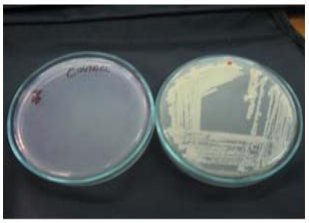
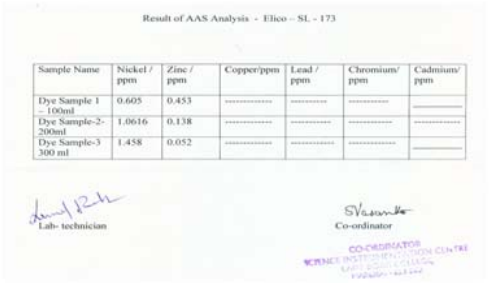


Plate: 1: Textile effluent collected from Salem district

Plate: 2 Organism showed dominant in various substrate concentration of textile effluent




Result of AAS Analysis - Elico - SL - 173

Sample Name	Nickel / ppm	Zinc / ppm	Copper/ppm	Lead / ppm	Chromium/ ppm	Cadmium/ ppm
Dye Sample 1 – 100ml	0.605	0.453	----------	----------	----------	----------
Dye Sample-2-200ml	1.0616	0.138	----------	----------	----------	----------
Dye Sample-3 300 ml	1.458	0.052	----------	----------	----------	----------

Lab- technician

Co-ordinator

CO-ORDINATOR

AAS for Heavy metal detection in textile effluent

Decolourization of Heavy Metals in Textile effluent:

Degradation of dyes in the present study in textile effluent was assessed and confirmed by the decolourization bioassay with least value of final colour. The decolourization increased gradually at 4-8 days monitored by UV spectrophotometer. (Plate-3).

Plate 3: Decoloration of textile effluent

Incubation Days	Control sample	1 ml	2 ml	3 ml	4 ml	5 ml
1st	0.000	0.312	0.401	0.457	0.513	0.537
2nd	0.000	0.207	0.311	0.379	0.416	0.493
3rd	0.000	0.175	0.289	0.377	0.412	0.483
4th	0.000	0.163	0.272	0.311	0.393	0.451
5th	0.000	0.147	0.233	0.285	0.361	0.412
6th	0.000	0.123	0.197	0.237	0.321	0.387
7th	0.000	0.133	0.172	0.193	0.291	0.327
8th	0.00	0.094	0.131	0.153	0.221	0.313

Reference

Hai FI, Yamamoto, Fukushi K (2006). Development of a submerged membrane Fungi reactor for textile wastewater, *Desalination* **192:** 315-32.

Hill, Perkins WE, Sandlin S, GS (1993). Removal of speciation of transition metal ions textile dyeing wastewater, *Text.chem. color* **25**: 26-27.

Junnarkar, Murty N, Bhatt DS, Madamwar NS (2006). Decolorization of di azo dyes Direct Red Slbye a novel bacterial consortium. World *J. Microbiol. Biotechnoloy* **2:** 163-168.

Maier, Kandelbauer J, Erlacher A, Cavaco-paulo A, Gubits A, G.M (2004)A new alkali .Thermostable azoreductase from *Bacillus SP*, Strain SF, *Appli. Envirom. Microbiol* **70:** 837-844.

Mcmullan, Meehan G, Cooeely C, Kirby A, Robinson Nnigam T, Banat p, Merchant IM, Smyth R (2001). Mini Review: Microbial decolorization and degradation of textile dyes, *Appl.Microbiol.Biotechnology* **56**: 81-87.

NaeemAli, Abdul Hameed, Safia Ahmed (2009). Physicochemical Characterization and metals by indigenous Bacteria, *Journal of Hazardous Materials* **164**: 322-328.

Nragu, Wong JO, Lawson HKT, Daniel GP(1998). Saturation of ecosystem with toxic metals in Sudbury basin, Ontario Canada, *Scien.Total.Environmental* **223** : 99-117.

Olukanni, Osuntoki OD, Gbentle AA, (2006).Textile effluent biodegradation potentials of textile effluent adapted and non adapted bacteria, Afr.J. *Biotechnology* **20**:1980-1984.

Pearce, Lioyd CI, Guthrie JR (2003). The removal of color from textile waste water using whole bacterial cell, *Dyes pigments* **58:** 179-186.

Sponza DT (2002). Necessity of toxicity assessment in Turkish industrial discharges (examples from metal and textile industry effluent), *Enviorn. Monit. Assess* **73**: 41-66.

Altschul, S. F., Madden, T. L., Scha¨ ffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25, 3389–3402.